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Inhibition of human glutathione *S*-transferase P1-1 by tocopherols and α-tocopherol derivatives

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Abstract

α-Tocopherol inhibits glutathione *S*-transferase P1-1 (GST P1-1) (R.I.M. van Haaften, C.T.A. Evelo, G.R.M.M. Haenen, A. Bast, Biochem. Biophys. Res. Commun. 280 (2001)). In various cosmetic and dietary products α-tocopherol is added as a tocopherol ester. Therefore we have studied the effect of various tocopherol derivatives on GST P1-1 activity. It was found that GST P1-1 is inhibited, in a concentration dependent manner, by these compounds. Of the compounds tested, the tocopherols were the most potent inhibitors of GST P1-1; the concentration giving 50% inhibition (IC_{50}) is <1 μM. The esterified tocopherols and α-tocopherol quinone also inhibit the GST P1-1 activity at a very low concentration: for most compounds the IC_{50} was below 10 μM. *RRR*-α-Tocopherol acetate lowered the V_{max} values, but did not affect the K_m for either 1-chloro-2,4-dinitrobenzene or GSH. This indicates that the GST P1-1 enzyme is non-competitively inhibited by *RRR*-α-tocopherol acetate. The potential implications of GST P1-1 inhibition by tocopherol and α-tocopherol derivatives are discussed. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Vitamin E; Tocopherol; Glutathione *S*-transferase; Inhibition; Human

1. Introduction

Glutathione *S*-transferases (GSTs) are a family of phase II detoxification enzymes that catalyze the conjugation of glutathione (GSH) to electrophiles. Numerous compounds are known to inhibit the GST activity. Recently, we reported that α-tocopherol is a potent inhibitor of the P1-1 isoform of GST [1].

Vitamin E is a generic name for all tocol and tocotrienol derivatives. The antioxidant activity is the most studied biological activity of these compounds discovered thus far, although other activities have also been found [2,3].

The tocopherols can be viewed as consisting of a chroman head consisting of two rings (one phenolic and one heterocyclic) and a phytyl tail. The phytyl tail has three chiral centers (at positions 2, 4′ and 8′), making a total of eight stereoisomeric forms possible (Fig. 1). The phytyl chain of naturally occurring tocopherols (α-, β-, γ-, and δ-tocopherol) has the *RRR* configuration. A synthetic tocopherol usually is a mixture of approximately equal amounts of the eight stereoisomers (*RRR*, *RRS*, *RSR*, *RSS*, *SRR*, *SRS*, *SSR* and *SSS*). This mixture is denoted as all-*rac*-tocopherol [4].

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In the antioxidant activity of tocopherol, the free aromatic hydroxyl group plays an essential role: the tocopherol can donate its phenolic hydrogen to lipid free radicals. Sequestration of this group inhibits the antioxidant activity but also makes the molecule less vulnerable to oxidation [4]. Therefore forms of α-tocopherol have been developed in which the free hydroxyl group has been blocked, e.g. by esterification of this alcoholic group with acetate or succinate. In vivo, the ester is saponified by an esterase revealing the antioxidant activity.

The aim of this study is to investigate whether tocopherols and α-tocopherol derivatives, other than α-tocopherol, can inhibit GST P1-1.

2. Materials and methods

2.1. Chemicals

The compounds tested were: *RRR*-α-tocopherol, all-*rac*-α-tocopherol, *RRR*-δ-tocopherol, *RRR*-α-tocopherol quinone, all-*rac*-α-tocopherol phosphate, *RRR*-α-tocopherol succinate, all-*rac*-α-tocopherol nicotinate, *RRR*-α-tocopherol acetate, all-*rac*-α-tocopherol acetate, arachidonic acid and trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid). All compounds were purchased from Sigma (St. Louis, MO, USA), except *RRR*-α-tocopherol quinone. This compound was synthesized from *RRR*-α-tocopherol by oxidation with ferric chloride, according to Cohen et al. [5]. 1-Chloro-2,4-dinitrobenzene (CDNB) and GST P1-1 (from human placenta) were also obtained from Sigma. GSH was obtained from ICN Biomedicals (Costa Mesa, CA, USA). All other chemicals were of analytical grade purity.

2.2. Assay of glutathione S-transferase activity

GST activity was measured as described by Mannervik and Guthenberg [6] with slight modifications. In short, the reaction of 1 mM CDNB with 1 mM GSH in 100 mM potassium phosphate, pH 6.5, at 37°C was monitored spectrophotometrically by recording the increase in absorbance at 340 nm. Effects of various concentrations of test compound on GST activity were determined. A stock solution of test compound was prepared in ethanol. The final concentration of ethanol in the incubation mixture was 1% v/v; this concentration of ethanol had no effect on GST activity. The mixture of the GST enzyme (0.0095 U/ml in buffer) with test compound was incubated for 2 min at 37°C before activity measurement. A correction for the spontaneous formation of the conjugate of GSH and CDNB in the absence of enzyme and in the presence of ethanol (1%) was made. To study the inhibitory mechanism of *RRR*-α-tocopherol acetate, substrate concentrations (CDNB or GSH) were varied. When CDNB was varied the GSH concentration was kept at 1 mM and vice versa. In these experiments the concentration *RRR*-α-tocopherol acetate was kept constant at 12.5 μM.

3. Results and discussion

All compounds tested (Fig. 1), except trolox and arachidonic acid, were found to inhibit human GST P1-1 activity towards CDNB at relatively low concentrations. An example of an inhibition curve at increasing concentration of test compound is given in Fig. 2 for *RRR*-α-tocopherol acetate.

The data in Fig. 1 show that both α-tocopherol and δ-tocopherol are very potent inhibitors of the P1-1 isoform of GST. The IC_{50} of α-tocopherol and δ-tocopherol are 0.7 μM and 0.8 μM respectively, indicating that there is practically no difference in potency between the α and the δ isoform. These two isoforms of tocopherol differ in the methylation pattern in the phenolic ring of the chroman head (Fig. 1) [4]. From this it can be concluded that methylation at positions 5 and 7 in the chroman head of tocopherol has no influence on the GST P1-1 inhibitory capacity.

From the results it also appears that sequestration of the hydroxyl group in the chroman head of the tocopherol molecule by acetate, succinate, phosphate or nicotinate lowers the GST P1-1 inhibitory capacity, although the compounds are still potent inhibitors (Fig. 1). Fig. 1 also shows that the oxidation product of α-tocopherol, α-tocopherol quinone (IC_{50} is 2.2 μM), is also active but has a lower inhibitory effect than the parent compound. The inhibitory potency of trolox was low; even at 50 μM no substan-

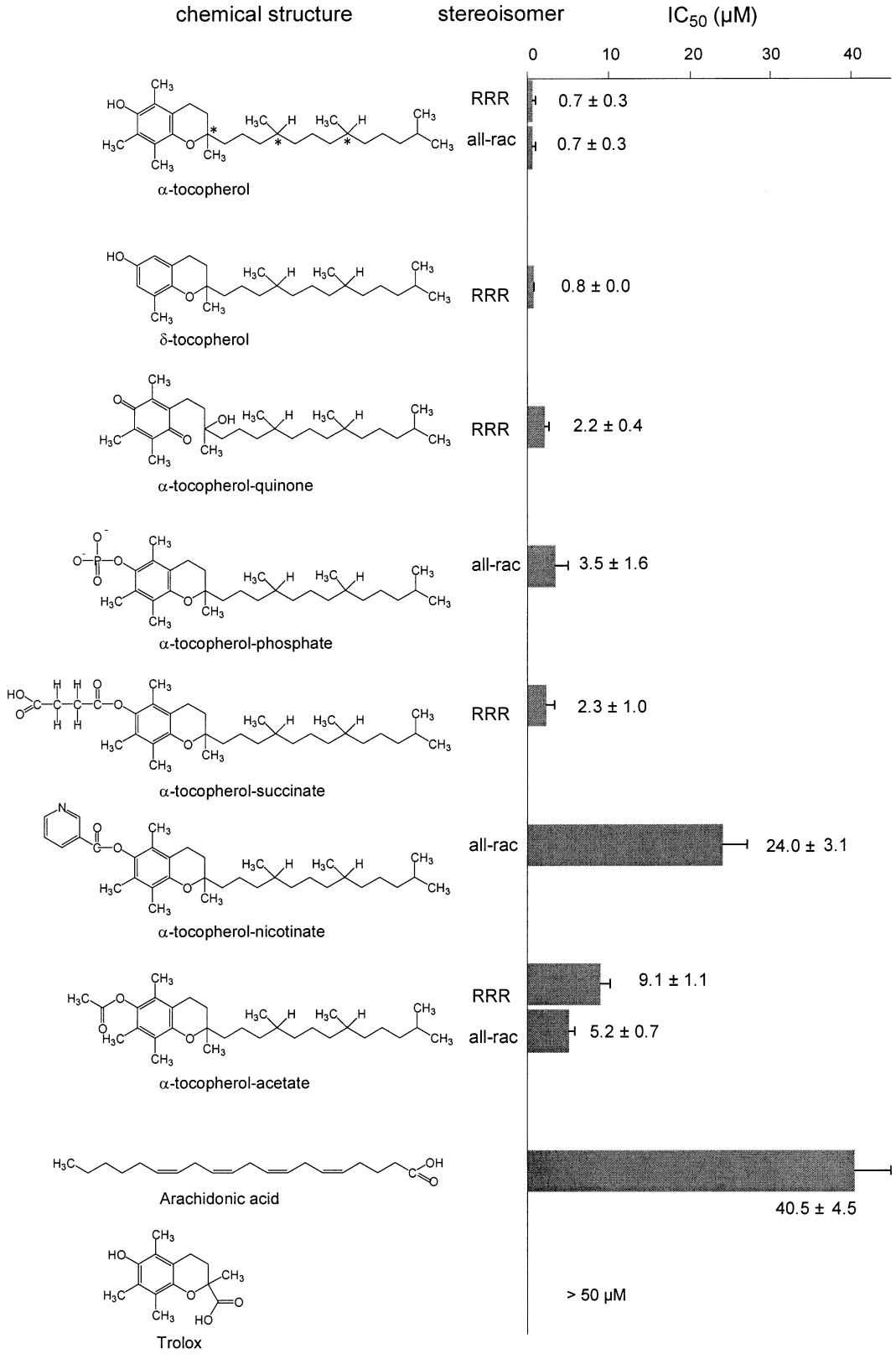

Fig. 1. Chemical structures of the compounds tested with the corresponding IC_{50} values. The three chiral centers in the phytyl tail are indicated with * in α-tocopherol. Of α-tocopherol and α-tocopherol acetate both the *RRR* and the all-*rac* forms are tested. IC_{50} values indicate means (±S.E.M.) of three independent experiments each performed in duplicate.

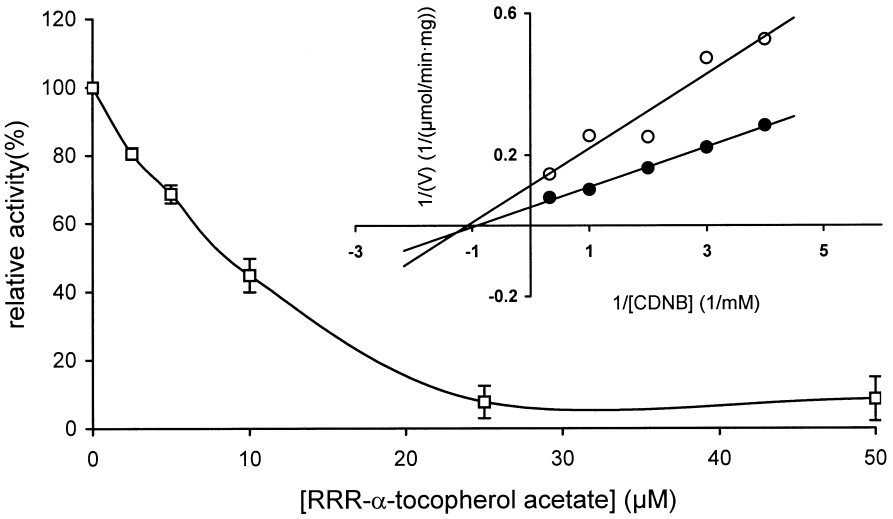

Fig. 2. Effect of *RRR*-α-tocopherol acetate on the activity of GST P1-1. The GST activity was measured by following the reaction between 1 mM glutathione and 1 mM 1-chloro-2,4-dinitrobenzene in 100 mM potassium phosphate, pH 6.5 at 37°C. Each point denotes the mean (±S.E.M.) of three independent experiments each performed in triplicate. (Insert) Lineweaver–Burk plot showing non-competitive inhibition of human placenta GST P1-1 isoenzyme towards CDNB by 12.5 μM *RRR*-α-tocopherol acetate. The K_m and V_{max} (mean ± range) of two independent experiments performed in triplicate for CDNB (●) are respectively 1.13 ± 0.23 mM and 19.71 ± 3.49 μmol/min·mg. In the presence of *RRR*-α-tocopherol acetate (○) these values are 0.94 ± 0.06 mM and 9.05 ± 1.45 μmol/min·mg.

tial inhibition of GST P1-1 activity was observed. This molecule has the same chroman ring as α-tocopherol; however, the phytyl chain is replaced by a negatively charged carboxylate group (Fig. 1). Based on these results we can conclude that the free aromatic hydroxyl group is not essential, while the phytyl tail probably has a major contribution to GST P1-1 inhibition. Thus other structural elements play a role in GST inhibition than in free radical scavenging in which the free hydroxyl group is important.

The IC_{50} values of the acetate esters of *RRR*-α-tocopherol and all-*rac*-α-tocopherol are 9.1 μM and 5.2 μM respectively. This implies that at least one stereoisomer of the all-*rac*-α-tocopherol acetate molecule inhibits GST P1-1 activity with higher potency than the *RRR*-α-tocopherol acetate. So the sterical orientation in the phytyl tail of the α-tocopherol acetate molecule seems to be important for the GST P1-1 inhibitory capacity. However, the *RRR*-α-tocopherol and the all-*rac*-α-tocopherol exhibit the same GST P1-1 inhibitory capacity; the IC_{50} of the *RRR*-α-tocopherol and the all-*rac*-α-tocopherol are 0.70 μM and 0.74 μM respectively. This indicates that the mean inhibitory capacity of all α-tocopherol stereoisomers is identical to that of *RRR*-α-tocopherol. In vivo, *RRR*-α-tocopherol has a greater bioavailability compared to the other stereoisomers [7] and the effect of this isomer might therefore still be the most important when a racemic mixture is given.

From the results it also appears that arachidonic acid inhibits the GST P1-1 activity with an IC_{50} value of 40.5 μM, much higher than for the tocopherols. So not all compounds with a long hydrophobic group inhibit the GST P1-1 activity at relatively low concentrations. Arachidonic acid is known to be a potent inhibitor of other GST isoenzymes. Mitra et al. [8] used a crude GST fraction from rat liver and found an IC_{50} of 17 μM, which is lower than the IC_{50} we found for GST P1-1 in this study.

For the compound *RRR*-α-tocopherol acetate the nature of the inhibition was also studied. GST activity was measured with variable concentrations of either CDNB (insert Fig. 1) or GSH (data not shown) in the presence of a fixed concentration *RRR*-α-tocopherol acetate. *RRR*-α-Tocopherol acetate lowered the V_{max} values, but did not affect the K_m (approx. 1 mM). This means that *RRR*-α-tocopherol acetate, like *RRR*-α-tocopherol [1], exhibits a non-competitive inhibition with respect to the substrates CDNB and GSH.

The most probable mechanism of GST inhibition by tocopherols, α-tocopherol derivatives and arachidonic acid is the induction of conformational changes of the enzyme. These conformational changes could be caused by volume increases and structural modifications of lipophilic regions that are present in the interface between the two monomers of the GST. Binding of a compound to this region can change the activity of the enzyme [9].

In conclusion, of all tested compounds the tocopherols were the most potent inhibitors of GST P1-1. However, the esterified tocopherols and the oxidized product also inhibit the GST P1-1 activity at a very low concentration (all α-tocopherol esters except all-*rac*-α-tocopherol nicotinate exhibit an IC_{50} value lower than 10 μM).

Mitchell and McCann [10] found that all-*rac*-α-tocopherol is a complete tumor promoter in mouse skin and Henderson et al. [11] showed that mice lacking the GST P1-1 have an increased risk for skin tumorigenesis. These results together with the fact that GST P1-1 is present in the skin [12] might be explained by the potent GST P1-1 inhibition.

When vitamin E is added to a product, e.g. a cosmetic product, mostly a tocopherol ester is used. This is due to the better chemical stability of the ester compared to the free tocopherol [13]. Most cosmetic products, containing a tocopherol ester, are applied to the skin. GST P1-1 is present mainly in many cells in the upper layers of the epidermis [14]. To estimate the maximal concentration of the tocopherol ester in the skin after application of a cosmetic product we assume that (i) all the tocopherol ester is transferred to the epidermis and (ii) the concentration in the epidermis is one tenth of the concentration in the cosmetic product. Indeed, Klain found that 4 h after dermal application of ^{14}C-labeled vitamin E, to human skin grafted athymic nude mice, the epidermis contained the most radioactivity. It was suggested that the skin can act as a reservoir for vitamin E [15]. Metabolism, elimination, distribution or accumulation due to frequent application of the cosmetic product is not taken into account in this estimation. Based on these assumptions it can be calculated that to reach a concentration of 10 μM of the tocopherol ester in the epidermis, a concentration inhibiting GST P1-1 more than 50%, 0.0005% w/v tocopherol ester in the cosmetic product would be sufficient. The concentration in most tocopherol ester containing cosmetic products exceeds this value by far. Concentrations up to 1% can be found; even products containing pure vitamin E are sold. This indicates that application of a tocopherol ester containing product on the skin probably results in a substantial inhibition of GST P1-1. Therefore, a potential tumor promoter effect may also be ascribed to α-tocopherol esters; however, further research is needed to substantiate this hypothesis.

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